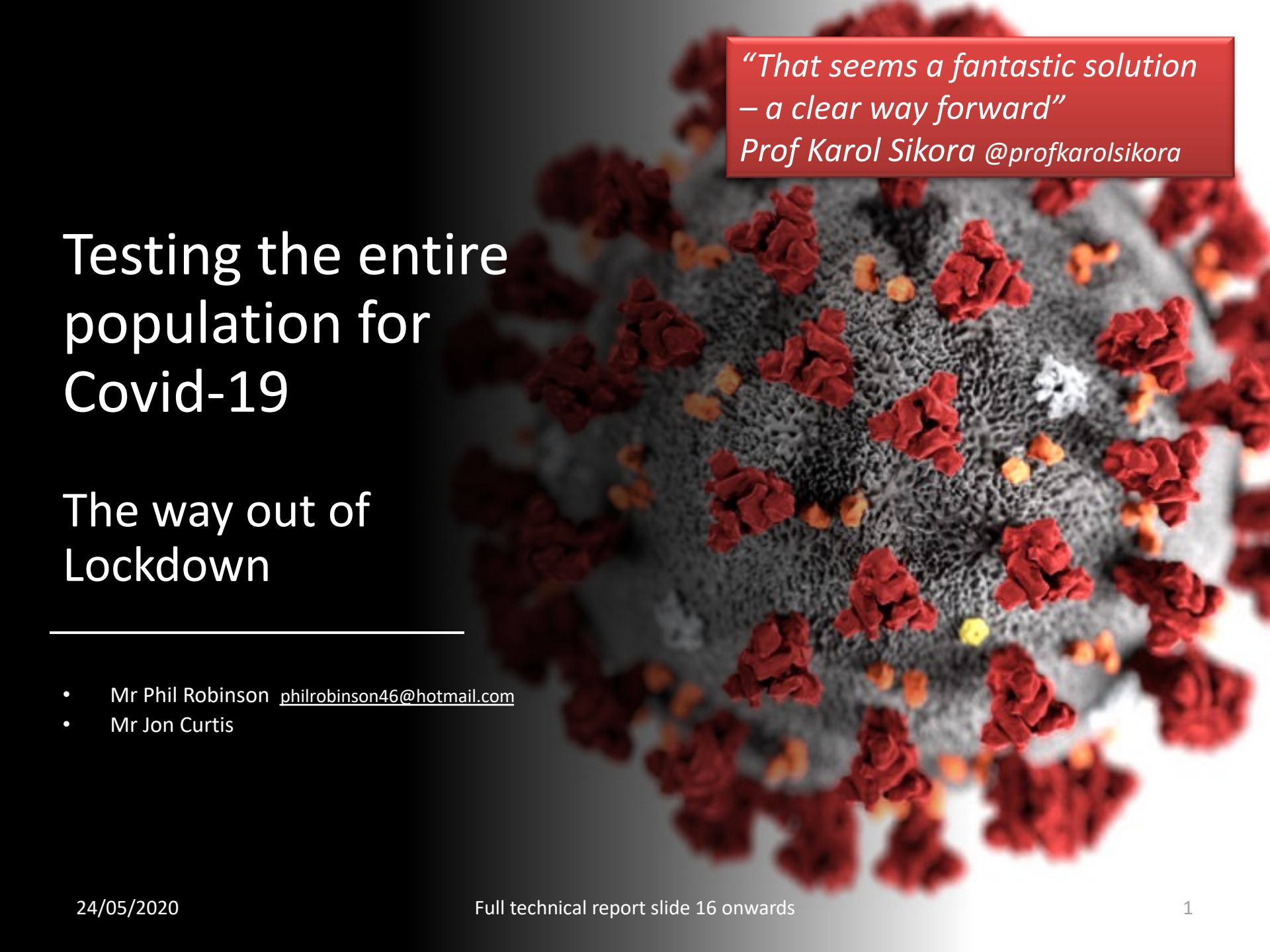


"That seems a fantastic solution – a clear way forward"
Prof Karol Sikora @profkarolsikora

Testing the entire population for Covid-19

The way out of Lockdown

- Mr Phil Robinson philrobinson46@hotmail.com
- Mr Jon Curtis

Testing entire Populations with the "PopMax" system

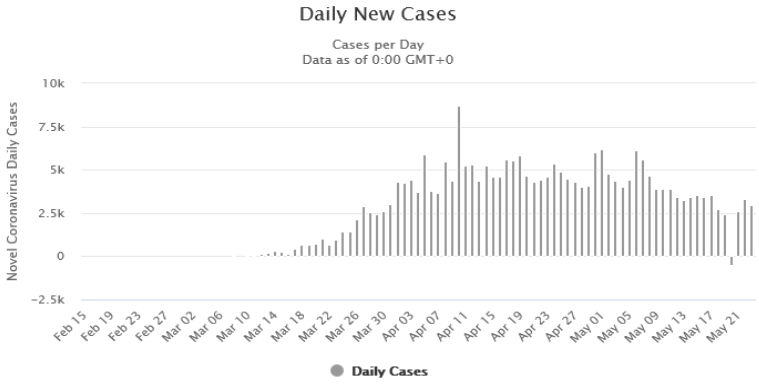
- From the WHO to SAGE, testing is seen to be fundamental to the UK coming out of the lock down successfully without a second wave.
- Current clinical Lighthouse lab testing takes 2 years to test whole population (based on 100,000 samples / day).
- The PopMax system can be used for both pooled testing for whole populations (the country) and individual testing of Hospital staff, Schools and Factories.
- This proposal uses a proven industrial genetic testing pipeline (already designed and developed by KBiosciences (UK), it is not new, just re-purposed). Proven on billions of samples.
- A single PopMax pipeline would enable 800,000 tests per day per lab at costs per test far below Lighthouse Megalab running costs.
- 3 labs across the UK would thus give a capacity of 65 million tests per month (approx). For the purpose of this presentation this system is being termed 'PopMax'. Details to follow.
- The pooling of samples by household is a significant multiplier in this strategy

Why do we need the PopMax system?

- Asymptomatic people transmit virus and do not get tested. We need to find them to contain the virus spreading again.
- People showing mild symptoms transmit virus but do not submit for testing. We need to find them to contain the virus spreading again.
- Using 100k tests per day with the current government strategy and contact tracing leaves the country exposed to the risk of a wave of new infections.
- Limited testing makes the R value a guess. Without population testing there is no ability to speedup/slow down lock down regionally.
- PopMax testing and contact tracing will allow lockdown to be lifted confidently, sooner saving lives and the economy.
- PopMax testing is not a clinical diagnosis it is a “stay at home” diagnosis, and could potentially clear the country of Covid-19.

Economic Cost of COVID-19 Lockdown

- Current estimates of Coronavirus Lockdown is £2.4 billion a day.
- 140,000 firms with furloughed workers.
- Two months of lockdown has been partially effective, but trajectory is stalled. The R value is still only just below 1.0
- Solution needed.



How do we test the entire population?

- To achieve 65 million samples processed per month requires 3 x PopMax systems installed across the UK. We would propose that they are on the same site as the current Lighthouse labs (Megalabs).
- The sampling of the population should be carried out per household in a pooling strategy. To be clear if four people live in a house then four swabs will be taken into a single sampling tube.
- This brings the number of samples to be run down to approx. 16 million per month.
- This is a pragmatic approach to pooling as a single positive sample leads to a house having to self-isolate.
- The PCR reaction will detect three Covid RNA amplicons of the viral genome to ensure specificity, plus an artificial RNA virus control that is spiked into the RNA extraction (this is consistent with WHO guidelines).

Technical details to consider in testing the entire population

- Testing the entire population should be seen as a screening exercise and should not be subject to current clinical PHE guidelines. *"Just stay at home"*
- To achieve the desired throughput a number of deviations from the PHE guidelines are proposed, however the system has already been validated with 100% concordance to current methods. The data is as accurate as current clinical methods.
- Please bear in mind that these deviations are just translations of protocols that have been used for billions of data points in industrial genetic typing for 20 years. The fact that the entire system is automated and software controlled will lead to higher quality data. At significantly reduced costs.

Costs, Timelines and Future

- Our test uses less reagents than current methods reducing cost considerably. It is conceivable that a single test in the lab (not including the swab) would be ~£1 or less, so to test the entire population would equate to 65million people / 4 (for each pool), thus £16 million per month.
- Capital setup cost for one lab is £2.07 million, so for all three labs it is just over £6 million.
- The PopMax system is a modified version of the KBioscience (now LGC genomics) SNPline system that has been installed in dozens of industrial Genetic testing labs throughout the world. Billions of data points have been generated to date.
- The PopMax system could be up and running in 6 weeks at the Milton Keynes site. The other sites would follow in the following four weeks.
- If the government decides to go forward with this proposal a system would exist that could carry out real time Covid or new virus surveillance (it can detect any virus) of the whole UK which could be rolled out globally.

PopMax is in step with current Gov strategy

"DHSC Coronavirus (COVID-19)
Scaling up our testing programmes"

- Pillar 1: NHS swab testing for those with a medical need and the most critical key workers – this would be fulfilled by PopMax

- Pillar 2: Commercial-swab testing for critical key workers in the NHS, social care and other sectors - this would be fulfilled by PopMax

- Pillar 3: Antibody testing to help determine if people have immunity to coronavirus

- Pillar 4: **Surveillance testing** to learn more about the disease and help develop new tests and treatments - this would be fulfilled by PopMax

- Pillar 5: Diagnostics National Effort to build a mass-testing capacity at a completely new scale - this would be fulfilled by PopMax

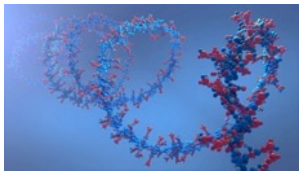
Carrying out testing on the whole population is impossible with the current government format.

PopMax offers mass testing of the entire population now.

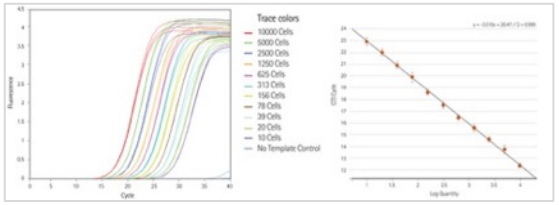
Every step of the PopMax process has been worked out

How does current Covid-19 testing work ?

A Swab is collected



Viral RNA is extracted

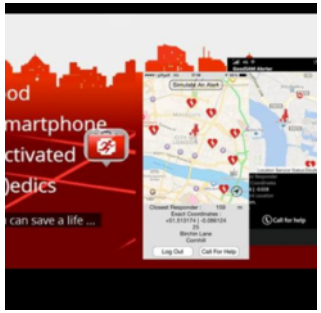


Real-time PCR is performed to detect virus

Data is analysed and test results are reported

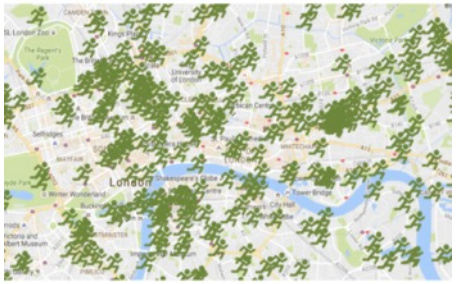
- Popmax also enables this to be carried out 200,000 times a day per lab
- The current UK Megalab setup requires huge numbers of volunteer staff.
- The current data analysis is not carried out by the Megalab, it is overly complicated, costly and slow.
- This is helping the virus to spread.

HOW: Every step of the PopMax process has been worked out



Swabs would be delivered to homes by the 750,000 GoodSam NHS Volunteer network supported by Royal mail / Amazon

Individuals would then self swab and the swabs from each household would be placed into a single tube (this is called pooling)



Swabs would be collected from homes by the NHS GoodSam network supported by Royal mail / Amazon into bio hazard barrels with NO packaging (very important for speed in the lab) These are then sent to the lab for testing

HOW: Every step of the PopMax process has been worked out

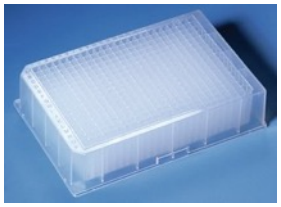
Bio Bins arrive into lab full of swab tubes, the solution in every tube must then be placed into many laboratory testing plates. Importantly there is <u>No packaging</u>

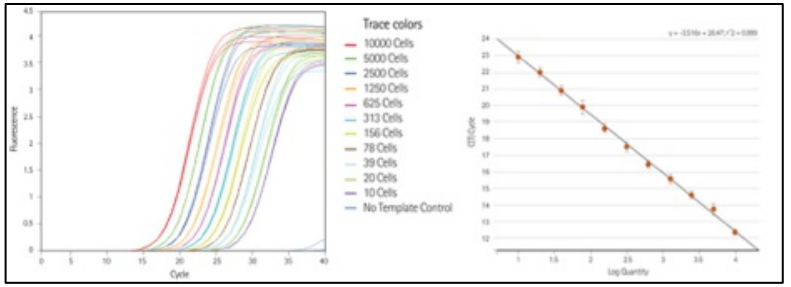
Q. How do you transfer 200,000 samples a day with minimal people?

A. <u>Use Industrial automation</u>

- Use a swab tube that holds the swab at the side (so it doesn't foul the liquid transfer robot)
- Use a laser to pierce the lid (so you don't have to take it off.)
- Use a step feeder to sort and present the tubes to a laboratory liquid transfer robot.

HOW: Every step of the PopMax process has been worked out



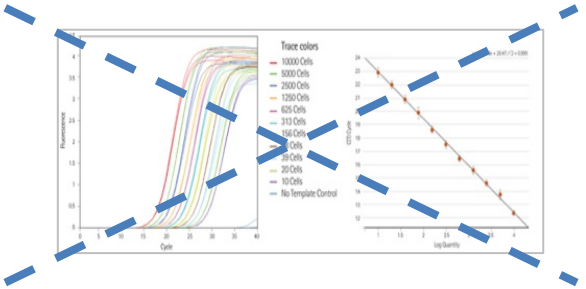


The swab solution is then purified ready for Viral detection

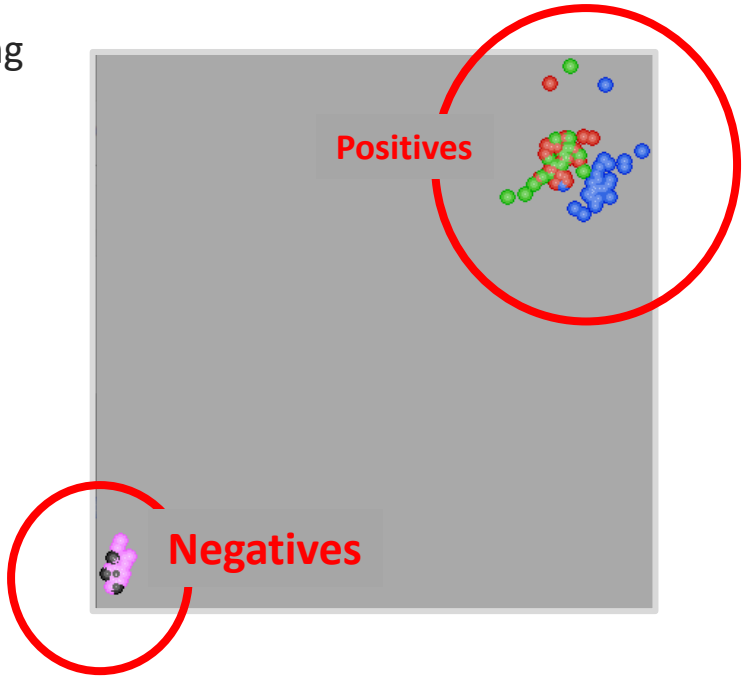
Q. How do you do this 200,000 times efficiently

HOW: Every step of the PopMax process has been worked out

A. Switch to a YES/NO test (like antibody or pregnancy tests), known as end-point.



- Current testing quantifies the amount of virus.
- This is not necessary.
- If you have the virus you have to self isolate.
- The actual test is the same, it is read at the end not during the test.
- The process is 36 times faster !!.
- The analysis is also up to 50 times faster.
- Faster data analysis and quicker reporting to individuals means LESS virus spread.
- This has already been validated by the authors on NHS Covid sample with 100% concordance



A statement from the authors

"We are a group of scientists who have come together in the face of the pandemic we find ourselves in. A number of us have volunteered to aid the Lighthouse Megalab testing setup to what could have been a world beacon of testing. But sadly we were just ignored on many many occasions. We believe that the current testing strategy is inadequate, cumbersome, ineffective and poorly managed. We are happy to expand on every issue in detail in front of any panel or establishment.

We have no commercial affiliations and just wish to help the UK and world deal with this global pandemic.

The full technical details of the proposal were sent to PHE, DHSC and government.

Bearing in mind that we might be considered the leaders in the world in ultra high throughput PCR based testing this has cost the country dearly"

The full technical details for this system can be found in below presentation

About the Authors

- Mr Jon Curtis is the co-founder of KBiosciences, prior to this he was at the Imperial Cancer Research Fund, Max Plank and GSK Technology development. He is the developer of mass industrial PCR technologies used at GSK, AZ, the Sanger Centre and across the world.

- Mr Phil Robinson is the co-founder of KBiosciences, prior to this he was at Amersham International and at GSK Technology development (Genetics dept). He is the inventor of KASP which has been used in billions of genetic tests to this day. E-mail – philrobinson46@hotmail

PopMax technical

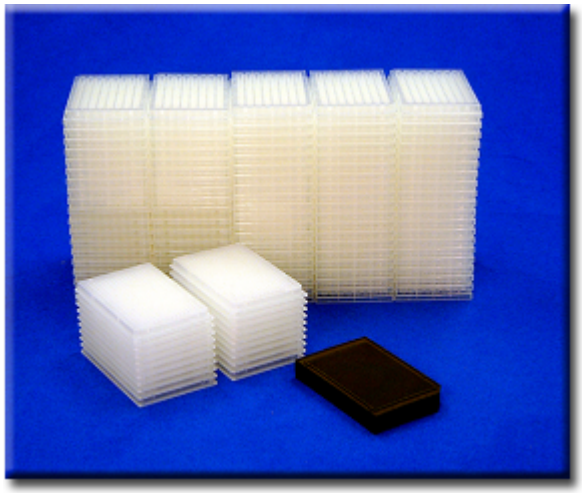
- Current lab processes
- Tube distribution
- Tube handling
- RNA extraction
- End point process
- Data handling

Current Lab process

Samples are received in large tubes, that are hand de-capped, then transferred to 96 well plates. These 96 well plates are then processed to purify the RNA from the virus. The RNA is then PCR amplified to detect the viral DNA, again in a 96 well plate.

The lab process can easily be sped up hugely by miniaturisation. This had been done by many scientists, but perhaps the experts in the field of genomics miniaturisation on an industrial scale are the team from KBiosciences (which was acquired by LGC genomics).

This allows the throughput to increase enough to test the whole population. It is not necessary to miniaturise to 1536 well plates for Covid testing as the numbers of PCR reactions is relatively small, 384 well plates are sufficient.



10,000 wells

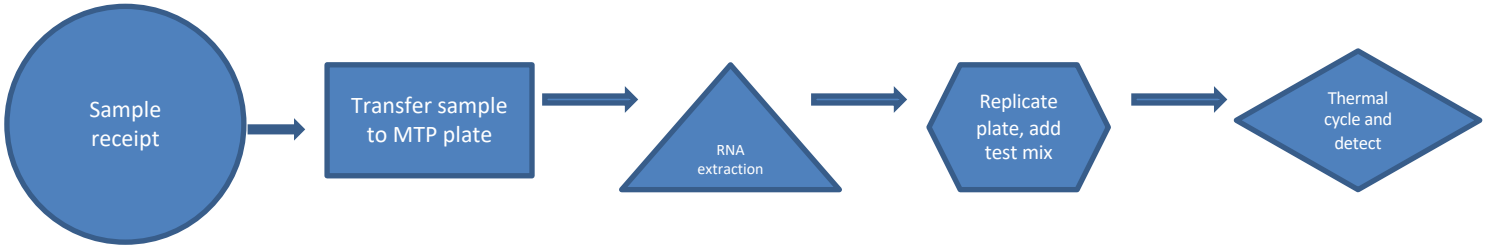
100 x 96 well plates

24 x 384 well plates

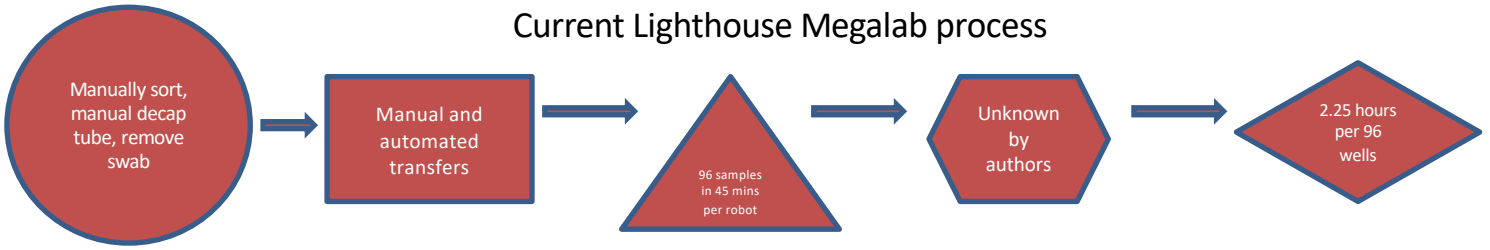
6 x 1536 well plates

What does the PopMax system look like?

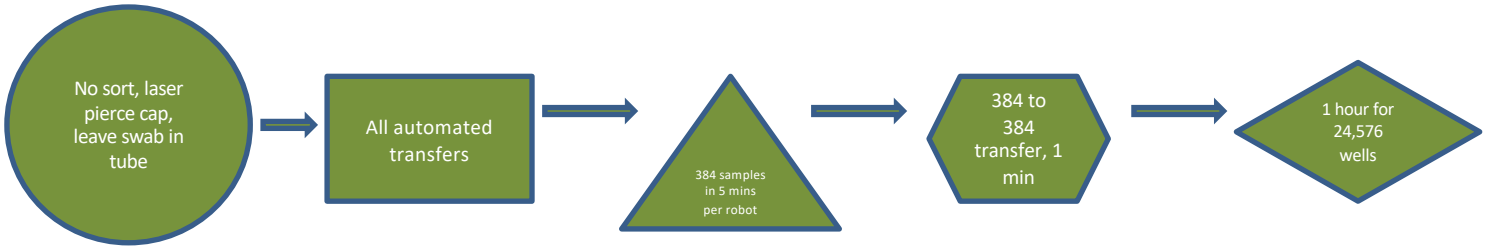
The system can be viewed as a pipeline, ie a number of pre existing instruments performing specific tasks in a formatted order



Current Lighthouse Megalab process



Proposed PopMax process



Swab Tube distribution collection to and from Houses/people

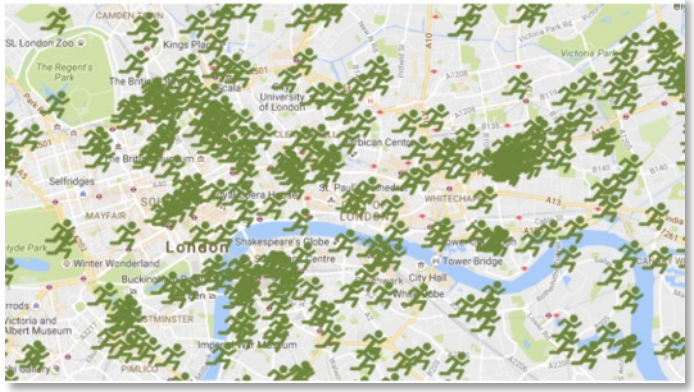
Our proposal is to utilise the existing

750,000 vetted NHS volunteer network

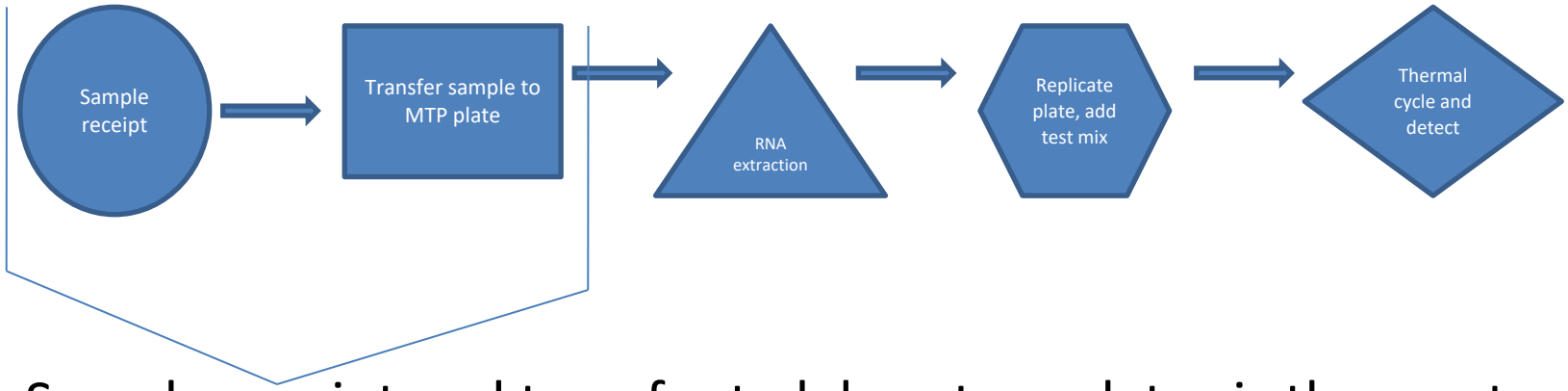
GoodSam, supported by Royal

mail/Amazon

https://testingmethods.crowdicity.com/post/3252460



What does the PopMax system look like?



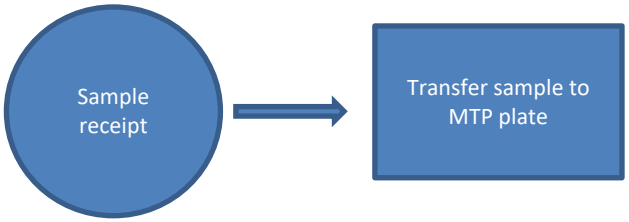
Sample receipt and transfer to laboratory plates is the most labour intensive part

The PopMax pipeline would transfer directly to 384 well plates
for the RNA extraction

A innovative solution utilising a novel swab tube, step feeding, laser piercing and direct liquid handling access is presented.

Sample Handling – The Problem

What does the PopMax system look like?



How to get 200,000 swab/saliva tubes into 384 (or 96) well plates ready for RNA extraction?

At present, a single UK Lighthouse lab can't cope with over 50,000 tubes per day.

The bottle neck is tube processing. A significant number are processed by hand !!!

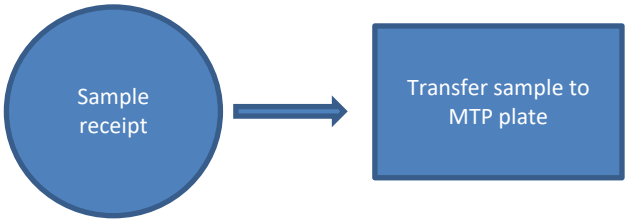
Tubes come into Lighthouse labs

Hand fed onto Tecan liquid dispenser
Caps have to be taken off, swab removed and barcodes need to be read

End result is highly automatable 384 (or 96) well plate

How to enable mass automation of sample transfer from tubes containing swabs?

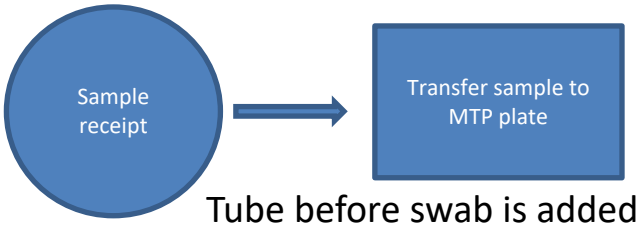
What does the PopMax system look like?



- The issue with automating is that the swab interferes with access to the sample for any automation
- 3Cr Bioscience have designed a tube to circumvent this
- The tube is designed to hold the swab away from the centre of the tube thus enabling clear access to the sample for an automated pipette tip
- The swab is placed into an inner tube which has a small drainage hole near the base to allow buffer to interact with the swab and for excess buffer to drain to the bottom of the main tube when upright
- A 3D video of the tube with and without swab can be found at https://3crbio.com/3dtube

Automatable Tube

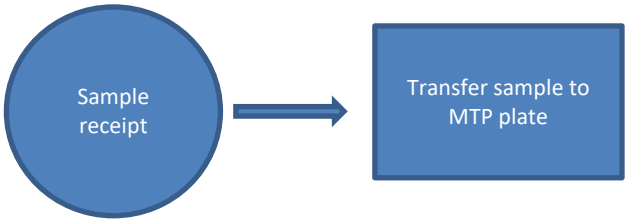
What does the PopMax system look like?



Tube before swab is added

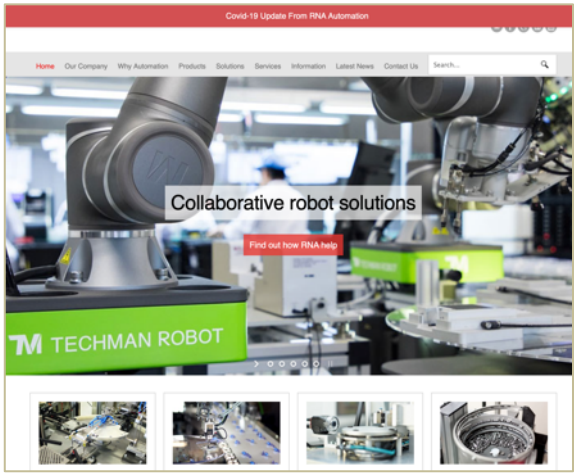
Tube with swab added

What does the PopMax system look like?



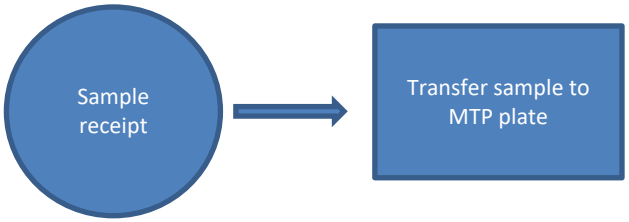
1. The swab tubes are placed into a hopper and a feeder is used to orientate and present the tubes one at a time in a linear fashion

We would use a step feeder, from RNA automation



The Solution – The Components

What does the PopMax system look like?



2. The tube needs to be de-capped and the swab removed

   Swab removal

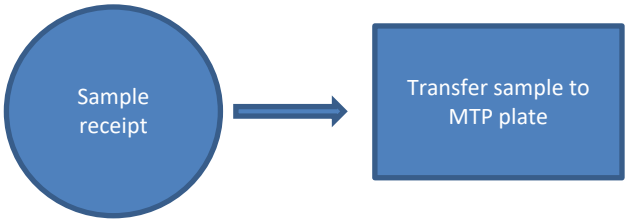
   We would use the tube designed by 3Cr bioscience, which ensures that the swab is attached to the tube side, so it does not impede access to the sample by the liquid handling robot. Without this tube, the very simple automation would NOT work.

   De capping

   This would be replaced by laser piercing, using a galvo-piercing laser. We would laser ablate a portion to sterilise then cut a flap that the laboratory liquid handler can push through.

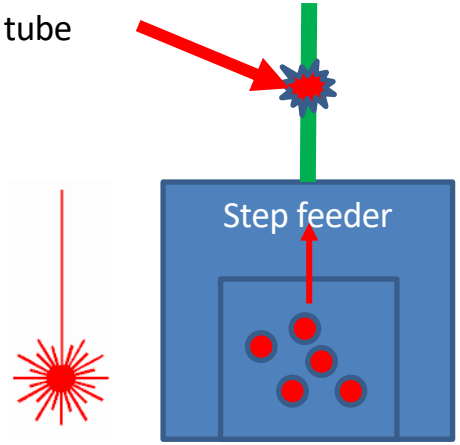
Non-Contact Laser cap piercing

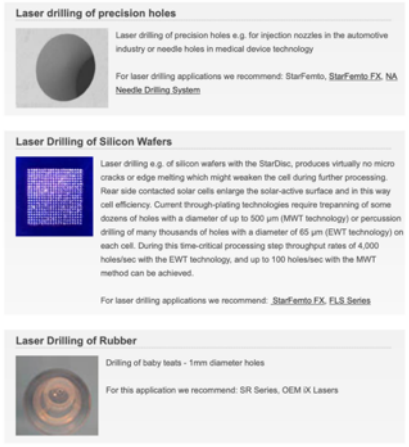
What does the PopMax system look like?

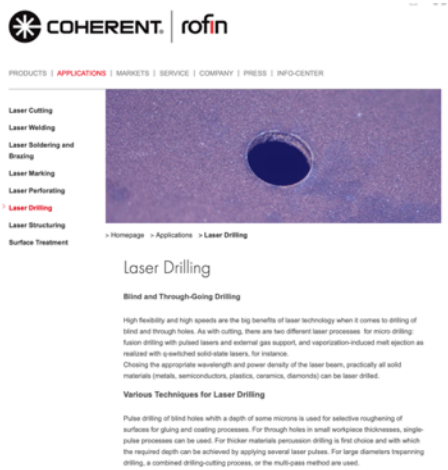


Galvonometer head - so no moving parts

Laser pierce tube

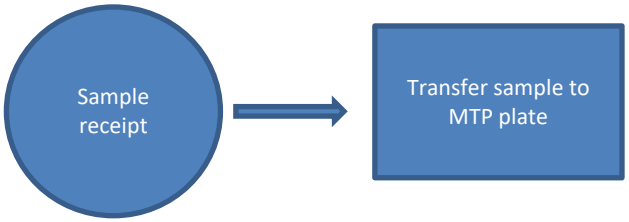






The Solution – The Components

What does the PopMax system look like?



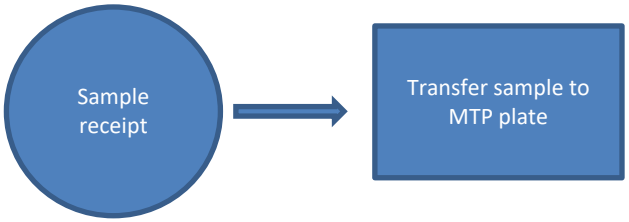
The tube then needs to be barcode read and placed onto the deck of a Tecan (or similar).

We propose not placing the tube onto the Tecan deck but aspirating from the automation. The Tecan then does not waste time in moving as much, the automation is simpler (and more reliable) and the barcode reading is done again not on the Tecan.

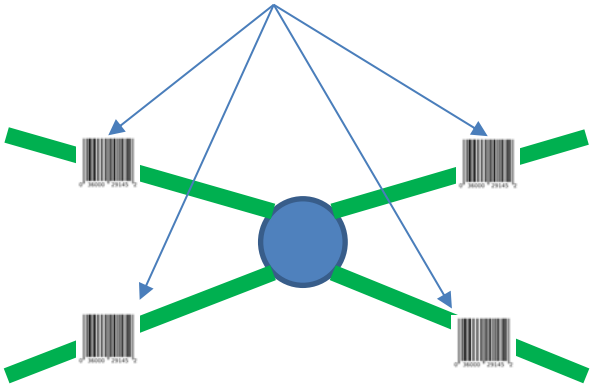
- Eliminates Tecan racks
- Eliminates de-capping
- Eliminates Tecan barcode reading
- Reduces human operation
- All liquid handling from feeder rail, passing tubes through Tecan bed into waste bin
- Significantly more efficient, less Tecan's and thus pays for itself.

The Solution – bar code reading

What does the PopMax system look like?



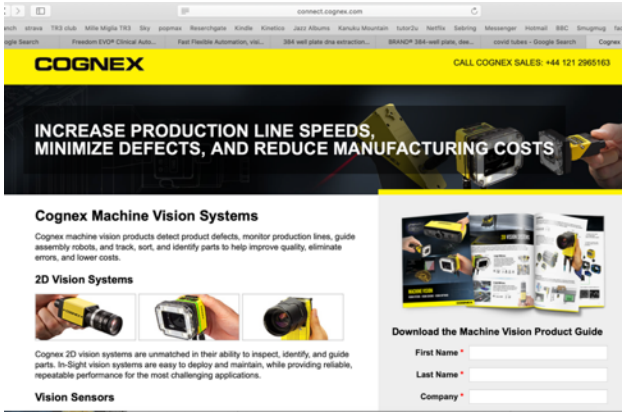
Bar code reading



COGNEX

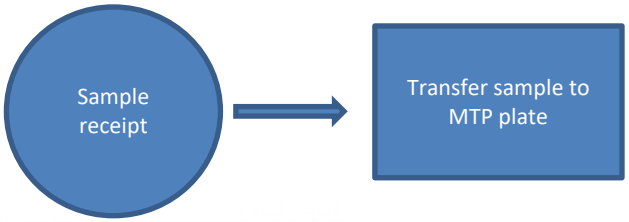
Tube bar code reader
Not on Tecan as saves time
X4 on input rails

https://www.cognex.com/en-gb



The Solution – tecan liquid handling

What does the PopMax system look like?



TECAN.

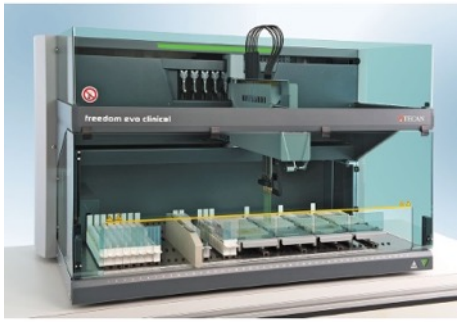
These are Tecan Liquid handling robots that are common throughout the world. The UK labs also use Hamilton and Integra liquid handlers.

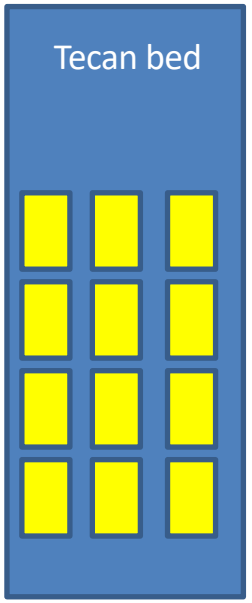
Automation drivers are available for Tecan liquid handlers as well as the other brands mentioned above, some examples are listed below

https://sila-standard.com/dipitems/tecan-evoware-driver-sila-2/

https://paa-automation.com/products/overlord-device-drivers/

http://www.biocompare.com/9991-Automated-MultiPurpose-Workstations/237025-Freedom-EVOwarereg/

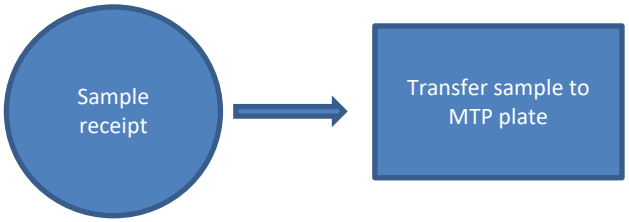




Current Tecan's at Lighthouse labs UK

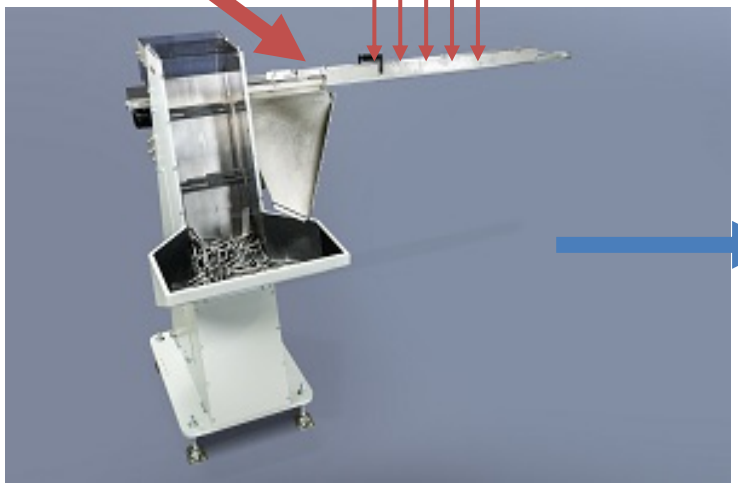
The Solution – Single Feeder to Tecan

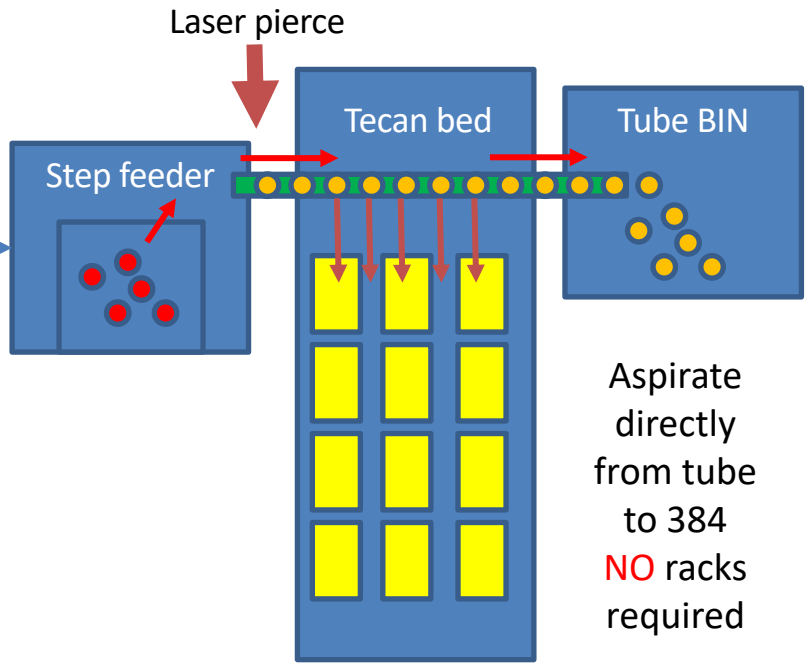
What does the PopMax system look like?



Laser pierce cap

Aspirate directly from linear feeder on bed of Tecan

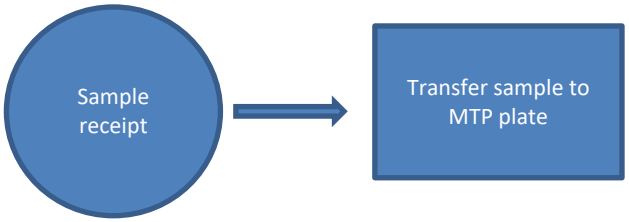




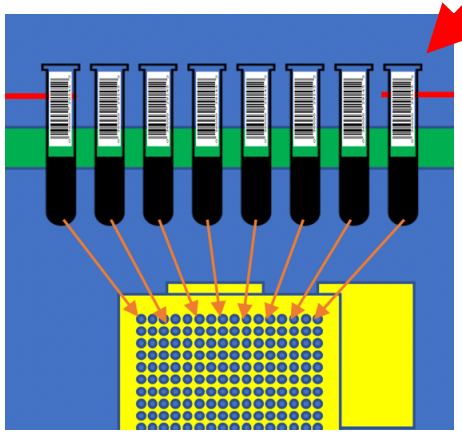
Aspirate directly from tube to 384 NO racks required

The Solution – liquid handle from feeder

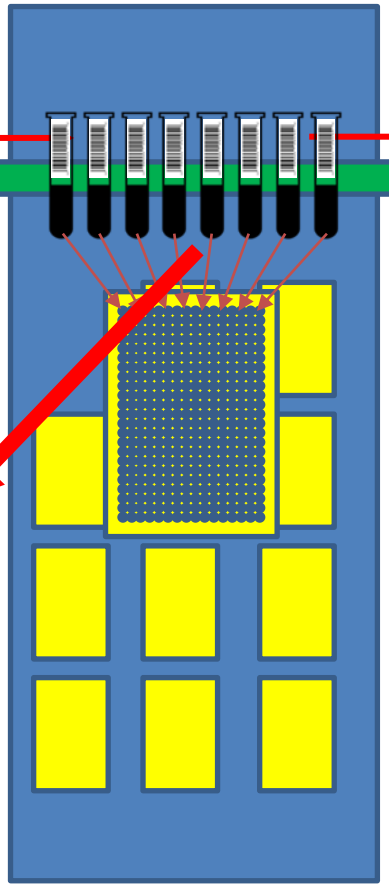
What does the PopMax system look like?

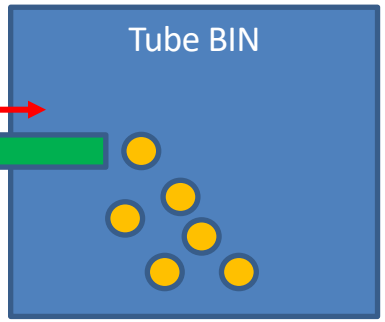


Tubes from Hopper
Laser pierced
Barcode checked



By the time the tubes get to the Tecan, 8 tubes are presented
All have been bar code checked post-hopper and laser holes cut in lids so no requirement to de-cap



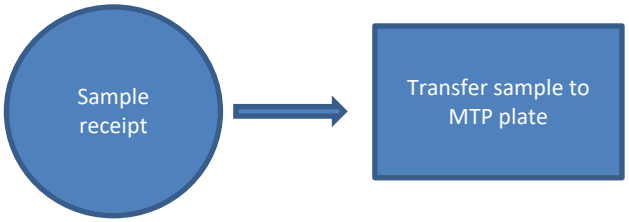


Aspirate directly from tube to 384
NO Tecan tube to place on bed or remove racks

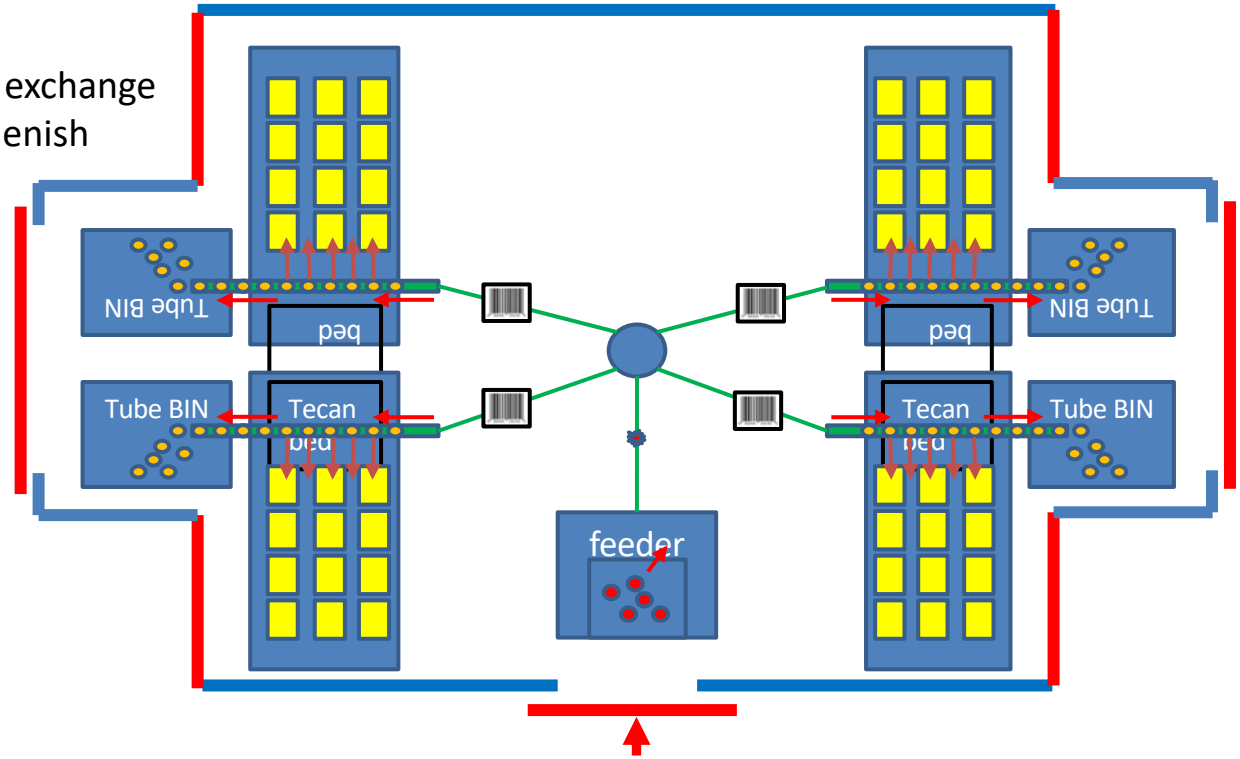
Whole bed can be taken up with 384 destination plates and tips
NO Tecan tube racks
NO barcode read on Tecan

4 x Tecan Sample handling Cell

What does the PopMax system look like?



384 plate exchange
& Tip replenish



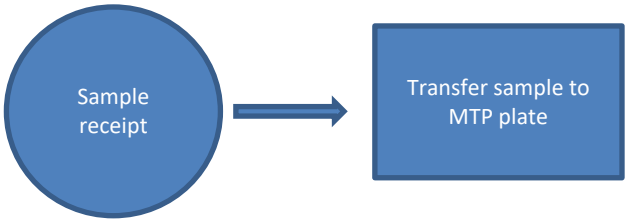
Single entry tubes out

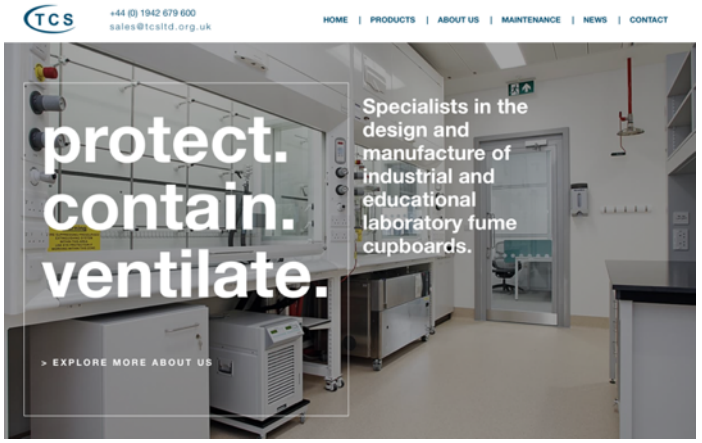
entry points

Single entry tubes in

4 x Tecan cell would be contained in a Safety Cabinet

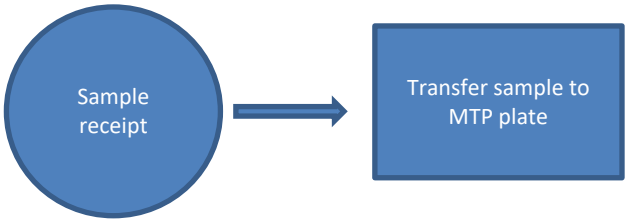
What does the PopMax system look like?





4 x Tecan Cell Throughput Calculations

What does the PopMax system look like?

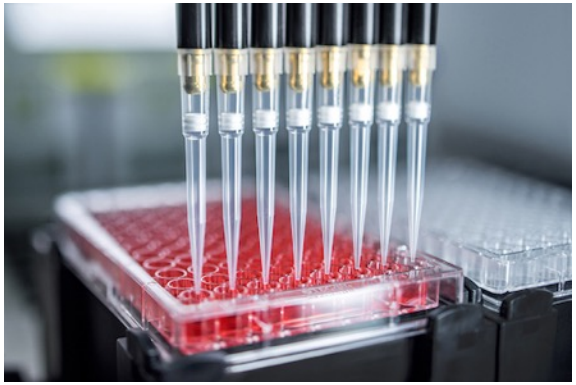


Assuming a 15 sec cycle time for a Tecan to transfer the solution to the 96/384 well extraction plate

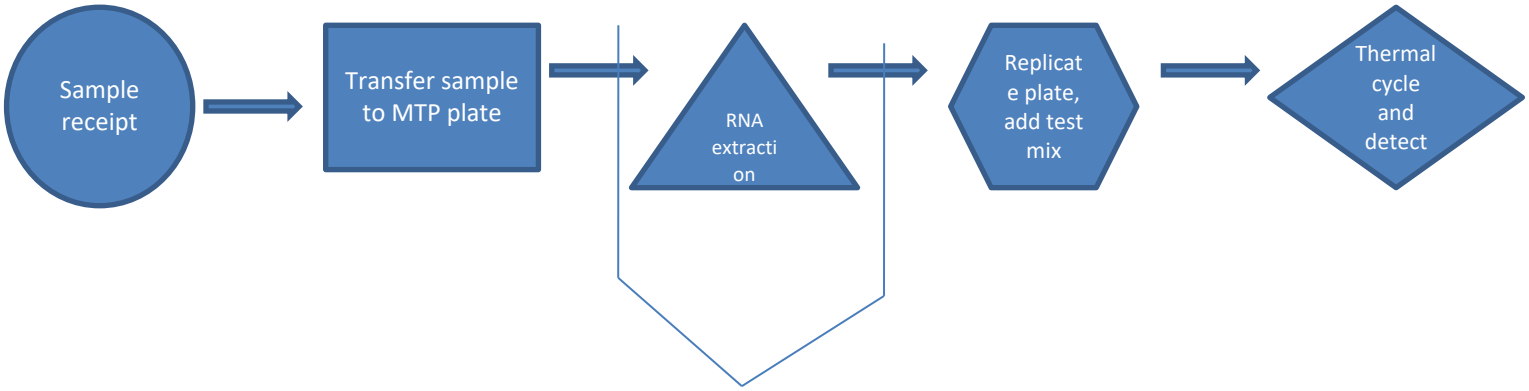
In a 24 hour cycle 184,320 tubes could be transferred to 96/384 plates per day per 4 Tecan cell

If pooled samples x4 = 737,280 population tests per day

15 sec cycle time
Pick up 8 tips,
Aspirate 8 tubes
Dispense 8 wells 96/384
Dispose 8 tips



What does the PopMax system look like?

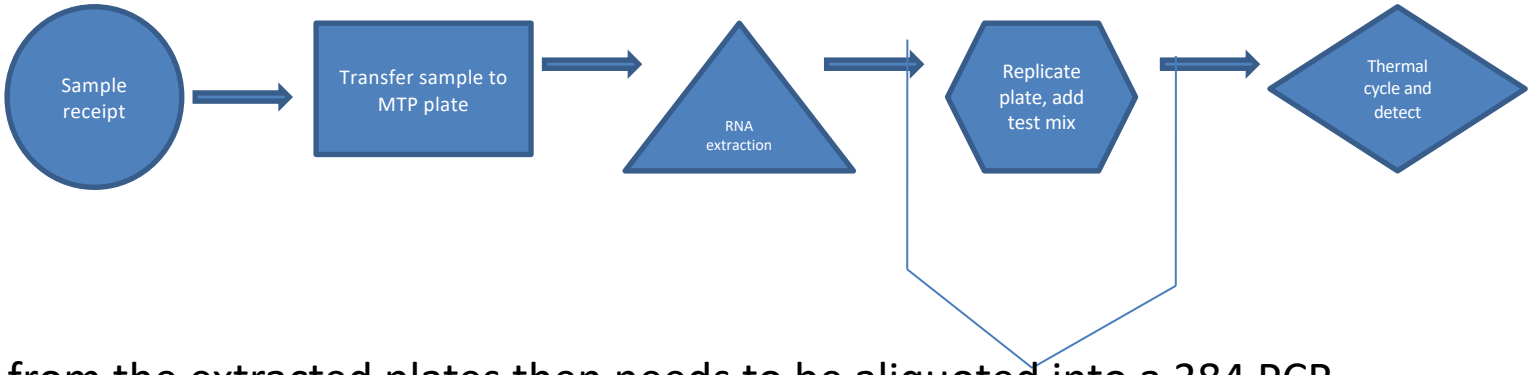


Ultra fast but high-quality viral RNA extraction is required.

This has been demonstrated by LGC genomics in 384 well plates.
It is commercially available.

Throughput calculations for 520 x 384 well plates (200,000 samples) using 2 x automated Heat sealer and stacker would be 5 hours.

What does the PopMax system look like?

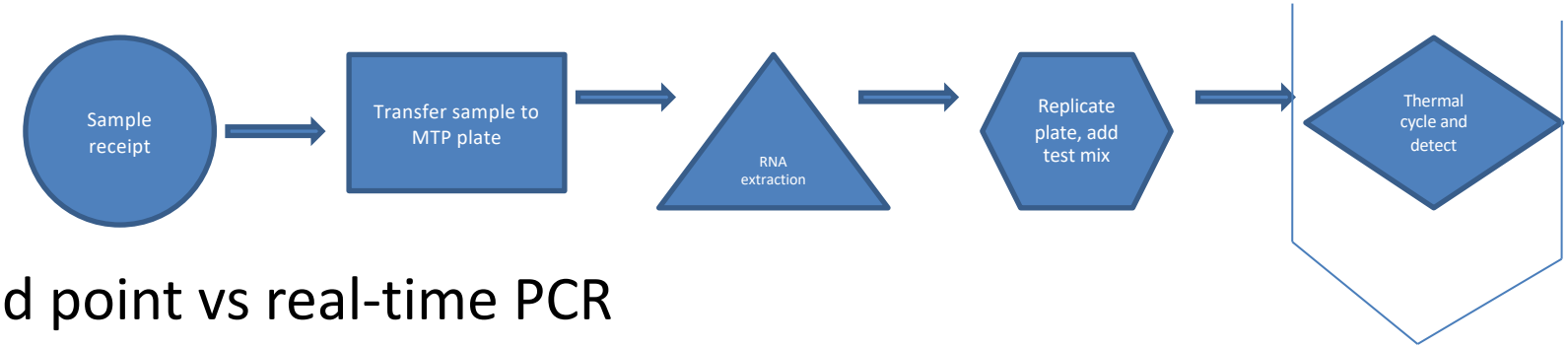


RNA from the extracted plates then needs to be aliquoted into a 384 PCR plate

The Replikator 384 pipetting robot carries this out with automated tip changing.

2 x RepliKator robots would produce the 520 plates per day required.

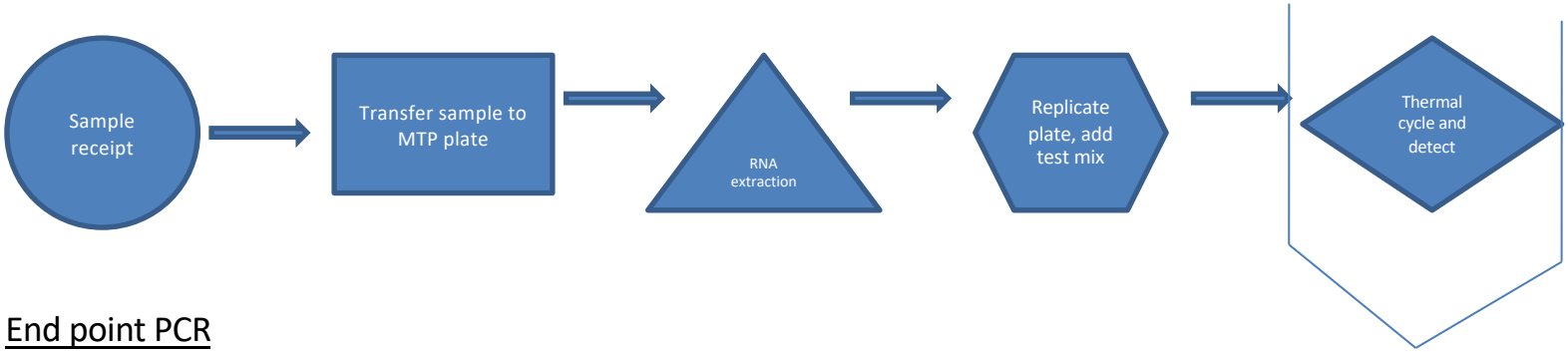
What does the PopMax system look like?



End point vs real-time PCR

- Current Megalab processes use real-time PCR for the detection of virus.
- Real-time PCR quantifies virus in the extracted sample. Quantification is not necessary. Indeed the information is NOT used.
- A test is reported as either positive or negative, therefore why is real-time PCR used? Running real-time PCR reactions in high throughput requires many many expensive machines. It is rate limiting. Additionally, the data analysis required is complex and labour intensive. This slows testing times down resulting in increased virus spread.
- End-point PCR tests whether an individual does or does not have the virus. This is all that is needed.
- End-point PCR can test hundreds of thousands of samples very quickly with upto 50 times less machines.
- Equally as important is the data analysis, which is also upto 50 times faster, meaning quicker results to the individual and again LESS viral spread.

What does the PopMax system look like?

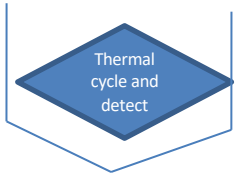


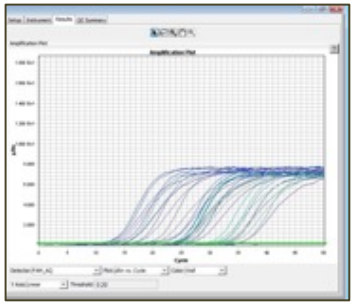
<u>End point PCR</u>

The ability to thermal cycle large numbers of plates was solved in the 1990's, using a robot water bath thermal cycler. These are used across the world and have carried out billions of reactions to date. A HC-64 robot is capable of 24,576 reactions in an hour.
3 x HC-64 robots per lab would fulfil the capacity requirements.

Once thermal cycling is complete the presence or absence of virus is determined with fluorescent dyes (it is exactly the same reaction used as in real-time PCR). A plate reader with associated stacker such as a BMG Pherastar will read a 384 well plate in 1 min. Three of these instruments would easily satisfy the throughput requirements.

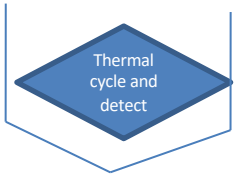
Current Lighthouse rtPCR throughput - per hour 13,440





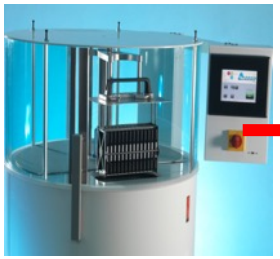
Data handling is Currently outsourced requiring complex machine learning.

Our End Point PCR 384 proposal - 69,920 per hour



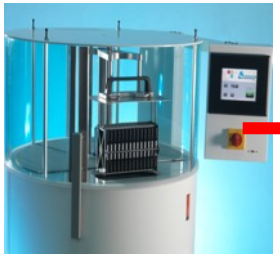
Load Hydrocyclers
Load plate stacker BMG Pherastar. Faster than QuantStudio
In house data scoring using Kraken LIMS

Hydrocycler 64



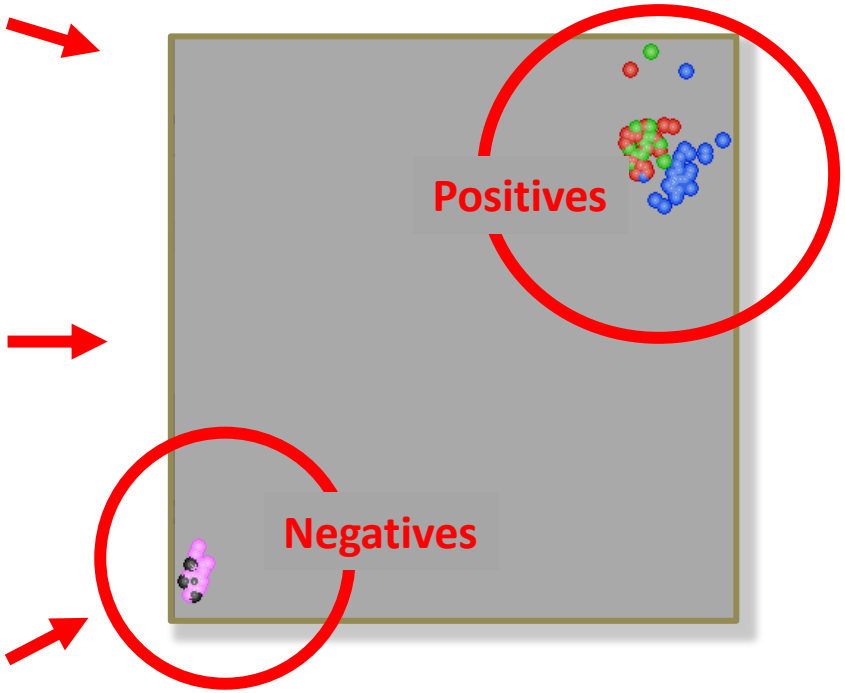
BMG Pherastar

Hydrocycler 64



BMG Pherastar

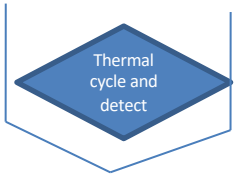
Hydrocycler 64



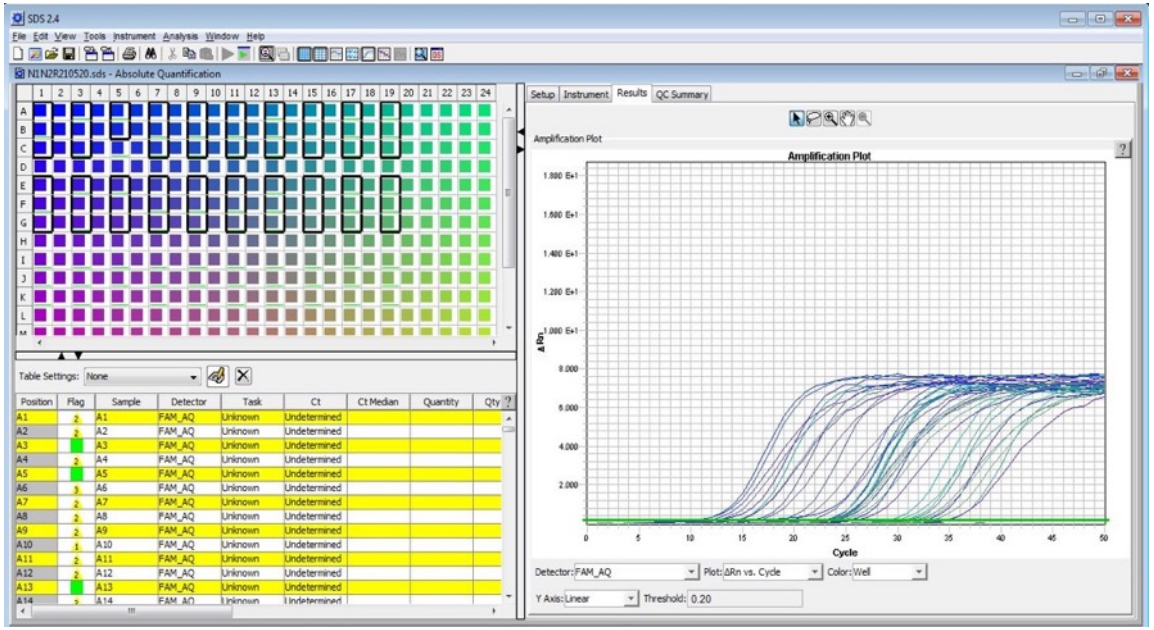
This INCLUDES data Analysis

Full technical report slide 16 onwards

End-Point Analysis - validation



The problem – ensure that weak positives are amplified to same degree as strong positives (ultimately this is sensitivity)



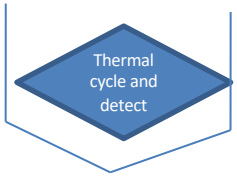
A number of previously tested positive and negative samples were obtained from a UK NHS testing laboratory.

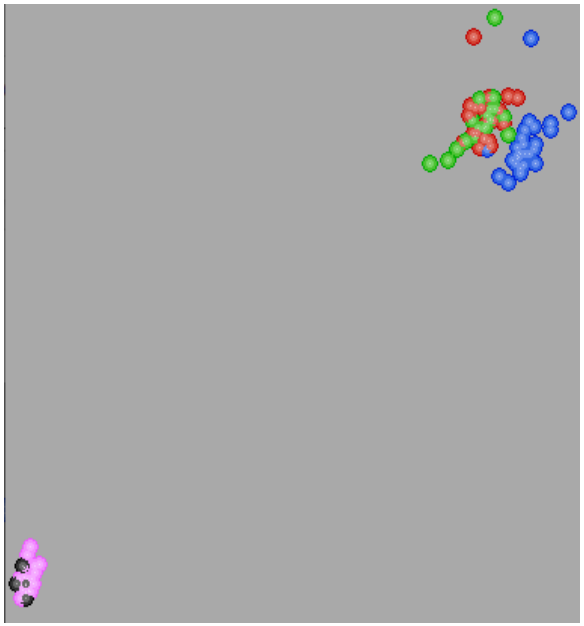
These were tested with an optimised ProbeSure™ Covid19 RT-PCR end-point mix from 3Cr Bioscience.

The reaction conditions were 1ul of RNA in a 5µl reaction (384 well plate). Ct values ranged from 12-37 and were comparable to the NHS testing lab which used 5µl of input RNA in a 25µl reaction (96 well plate)

Approximate 5-fold increase in sensitivity with ProbeSure™ Covid19 RT-PCR mix

End-Point Analysis Continued…



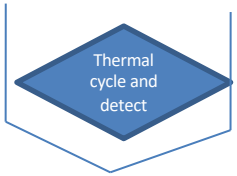


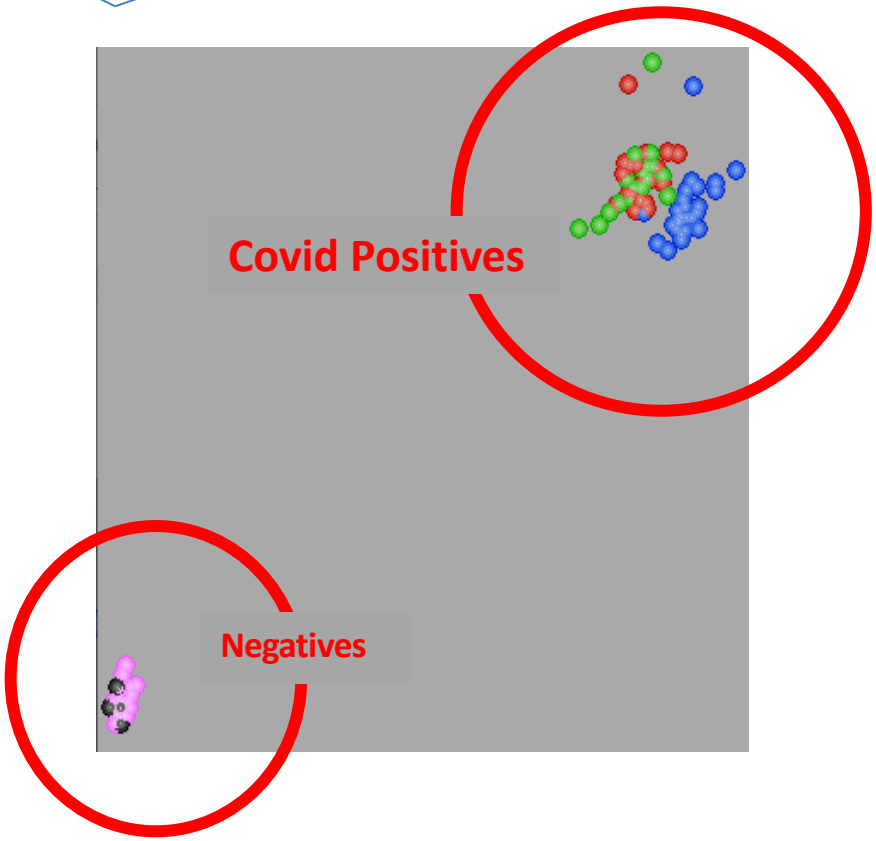
- The plate was then quantified on a Tecan plate reader
- Data is ROX normalised
- The green cluster contains the positive samples for the N1 Covid-19 gene
- The red cluster contains the positive samples for the N2 Covid-19 gene
- The blue clusters are the positive control for the RNAase P gene
- The pink cluster contains the negative samples
- The black samples are the negative controls
- Data is presented at 50 cycles (PCR end-point)
- Data is 100% concordant

ProbeSure™ Covid19 RT-PCR end-point mix is fully optimised for end-point analysis in 384 well PCR plates with higher sensitivity than UK testing lab mix.

Next step is to determine limit of detection in copies per ml

End-Point Analysis Continued…



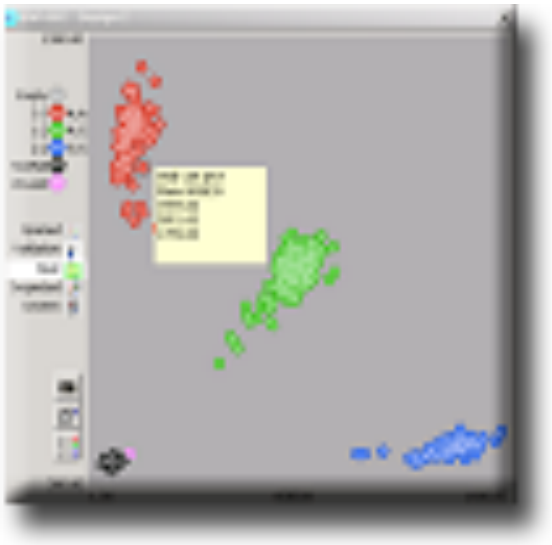


Data Analysis

One of the forgotten but, very important improvements that end-point Covid Analysis brings is that data analysis is extremely easy. It is a bottleneck in the UK labs due to the perceived need to analyse the curve of a real-time plot. This is not necessary with end-point data. One data analyst would easily score over 400 x 384 well plates per day (153,600 tests) using Kraken Software.

Informatics and Analysis

- The workflow of any laboratory must have a LIMS (laboratory Information Management system), that tracks every sample from beginning to end of the process. It must support barcode reading of every step.
- The Kraken LIMS system was developed for a process almost identical to the proposed PopMax system and is an off the shelf solution.
- It is capable of tracking billions of samples and enables the Covid-19 data analysis speed to be increased by up to 50-fold.



Kraken Workflow Manager

Costs, Timelines and Future

All cost are based on internal NHS PHE labs adopting PopMax protocol.

PopMax is not a company or service offering.
Popmax is just best practise.
All information is free to distribute and adopt.

Estimated capitol cost would be significantly cheaper for smaller labs.
Hospitals etc could re-purpose existing equipment

- Our test uses less reagents than current methods reducing cost considerably. It is conceivable that a single test in the lab (not including the swab) would be ~£1 or less, so to test the entire population would equate to 65million people / 4 (for each pool), thus £16 million per month.
- Capital setup cost for one lab is £2.07 million, so for all three labs it is just over £6 million.
- The PopMax system is a modified version of the KBioscience (now LGC genomics) SNPline system that has been installed in dozens of industrial Genetic testing labs throughout the world. Billions of data points have been generated to date.
- The PopMax system could be up and running in 6 weeks at the Milton Keynes site. The other sites would follow in the following four weeks.
- If the government decides to go forward with this proposal a system would exist that could carry out real time Covid or new virus surveillance (it can detect any virus) of the whole UK which could be rolled out globally.

Contact Details

- Phil Robinson – philrobinson46@hotmail.co.uk
- Jon Curtis – joncurtis7@hotmail.com

For further details on the automatable tube and/or ProbeSure™ Covid19 RT-PCR endpoint mix, please contact:

- Steve Asquith Managing Director steve.asquith@3crbio.com
- Dr John Holme Technical Director john.holme@3crbio.com
- Dr Nisha Jain Operations Director Nisha.jain@3crbio.com